Remarks

Claims 47-51, 53-64, and 73-79 are currently pending. Applicants acknowledge with appreciation that claims 53-57 and 73-79 would be allowable if re-written in independent form including all of the limitations of the base and any intervening claims.

Applicants request entry and consideration of the remarks into the record.

I. Summary of Examiner Interview

Applicants note with appreciation the courtesies extended during the interview on June 12, 2009, in which Examiner Long, Supervisory Examiner Woitach, and Applicants' representatives Nikolaos C. George and Colin A. Forestal participated (the "Interview"). During the Interview, the outstanding obviousness rejection pending in the Non-Final Office Action, mailed February 5, 2009, was discussed and the undersigned presented the reasons, detailed below, warranting favorable action. As indicated in the Interview Summary issued by the Patent Office, agreement was reached regarding these arguments.

II. The Rejections for Obviousness Should be Withdrawn

Claims 47-51 and 58-64 remain rejected under 35 U.S.C. § 103(a) for allegedly being unpatentable over Yazawa, *et al.* (Proceedings of the American Association for Cancer Research Annual Meeting , Vol. 40, pp. 88, 1999; "Yazawa") in view of Brown, *et al.* (US. Publication NO. 2003/0103952; "Brown"), and in further view of both Goshima, *et al.* (Biochimie, Vol. 72, pp. 207-214, 1990; "Goshima" and Claret, *et al.* (J. Mol. Biol. Vol. 273, pp. 93-104, 1997; "Claret"). For at least the following reasons, Applicants respectfully disagree with this rejection.

A. The Legal Standard

A finding of obviousness requires that "the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. §103(a).

In *KSR International Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 82 USPQ2d 1385 (2007), the Supreme Court stated that the following factors set forth in *Graham v. John Deere Co.*, 383 U.S.

1, 148 USPQ 459 (1966) still control an obviousness inquiry: (1) the scope and content of the prior art; (2) the differences between the prior art and the claimed invention; (3) the level of ordinary skill in the art; and (4) objective evidence of nonobviousness. *KSR*, 127 S.Ct. at 1734, 82 USPQ2d at 1388 quoting *Graham*, 383 U.S. at 17-18, 14 USPQ at 467. The Supreme Court affirmed that to find obviousness, it is "*important to identify a reason* that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does." *KSR*, 127 S.Ct. at 1741, 82 USPQ2d at 1396, emphasis added. Moreover, the relevant inquiry is whether the prior art suggests the invention and whether the prior art provides one of ordinary skill in the art with a reasonable expectation of success. *In re O'Farrell*, 853 F.2d 894 (Fed. Cir. 1988).

B. Yazawa, Either Alone or Combined with Brown, Goshima, or Claret Fails to Teach or Suggest the Claimed Invention

The Patent Office continues to contend that although neither Yazawa nor Brown teach using an HU promoter in *Bifidobacterium* to express anti-tumor genes, as recited in the pending claims, this deficiency is supplied by Goshima and Claret.

1. The HU Gene Does Not Fit The Patent Office's Description Of A Housekeeping Gene

With respect to Goshima, the Patent Office contends that the protein encoded by the HU gene is a histone-like protein and that Goshima teaches the existence of a HU-like protein in *Bifidobacterium*. The Patent Office further posits that, in eukaryotes, histones are housekeeping genes and promoters of housekeeping genes, *e.g.*, the β-actin promoter, are good candidates for use in expression vector systems because they can constitutively express heterologous genes at high concentrations. The Patent Office concludes that the *Bifidobacterium* HU protein is a housekeeping gene, the promoter of which would seem a good choice for a promoter in an expression vector system (*see* page 5, first paragraph of the Office Action mailed February 5, 2009; "Office Action").

Applicants first point out that Goshima only teaches the existence of a *histone-like* protein in *Bifidobacterium*. It is important to note that histone-like proteins are *not* histones

themselves and thus, even assuming that eukaryotic histones are housekeeping genes, it does not necessarily follow that prokaryotic histone-like proteins are housekeeping genes. Indeed, the homology between histones and histone-like proteins has been discounted by those of ordinary skill in the art (*see, e.g.*, Tanaka et al., 1993, J. Biochem. 113:568-572, "Tanaka," Reference C06, made of record herewith). In the first paragraph of the Introduction, Tanaka states that "HU is ubiquitous in the bacterial kingdom and wraps DNA...but is not homologous with eukaryotic histones." Further, in *E. coli*, the HU protein can be deleted without having any negative effect on the bacteria under normal growth conditions (*see, e.g.*, Wada et al., 1988, J. Mol. Biol. 204:581-591, Reference C07, made of record herewith; and Kano et al., 1990, Gene 89:133-137, Reference C08, made of record herewith), which would not be expected for a housekeeping gene. Moreover, the suggestion that the gene encoding the HU protein is a housekeeping gene is further minimized by the role of HU in DNA synthesis. HU seems to perform a niche role, *i.e.*, binding kinked DNA, and it has been postulated that HU is in fact an analogue of a eukaryotic protein that binds to kinked DNA (HMG-1) and **not** an analogue of histones (*see, e.g.*, Pontiggia et al., 1993, Molec. Microbiol., 7(3):343-350, Reference C09, made of record herewith).

Finally, Applicants point out that the HU gene fails to meet the main attribute cited by the Patent Office as belonging to housekeeping genes: constitutive expression. Unlike the constitutive expression observed with housekeeping genes, *e.g.*, β-actin, the expression of the HU protein in *E. coli*, as demonstrated by Claret (*see* Figure 1), is not constitutive at all. Rather, the *E. coli* HU protein promoters drive HU gene expression only during the logarithmic growth phase of the bacteria, and are found to be inactive at other times of the bacterial life cycle.

2. Goshima Does Not Teach That The *B. longum* HU Protein Consists Of A Homodimer Made From Monomers Like The *E. coli* HU-1 Subunit

As shown by Claret, the *E. coli* HU protein exists primarily as a heterodimer comprising two subunits (HU-1 and HU-2). HU-1 protein expression in *E. coli* is driven by a single promoter; whereas HU-2 protein expression in *E. coli* is driven by three separate promoters. The Patent Office alleges that Goshima demonstrates that *Bifidobacterium* HU "consists of a homodimer made from monomers like the HU-1 subunit of *E. coli* HU" (*see* page 5, second

paragraph of the Office Action), and thus concludes that one of ordinary skill in art would have every reason to believe that *B. longum* would have a single promoter (not three separate promoters) and act in a similar manner as the *E. coli* HU-1 protein promoter.

Applicants respectfully disagree with the Patent Office and point out that nowhere does Goshima state that the *Bifidobacterium* HU protein is similar to the *E. coli* HU-1 subunit. What Goshima states is that the *Bifidobacterium* HU-like protein HB1 "has similar properties to the **HU protein** in *E. coli* and is mainly present in *B. longum* as a homotypic dimer" (*see* Goshima at page 207, right column; emphasis added). Thus, Goshima is actually saying that *Bifidobacterium* HU protein, which comprises a homotypic dimer, has similar properties to the *E. coli* HU protein, which, as taught by Claret, can comprise a heterotypic dimer made up of two separate HU protein subunits (HU-1 and HU-2), a homotypic dimer made up of two HU-1 subunits, or a homotypic dimer made up of two HU-2 subunits. Indeed, Goshima's discussion of the *E. coli* "HU protein" always refers to the heterodimeric protein and all of Goshima's experiments that involve the *E. coli* HU protein use the heterodimeric protein. Clearly, Goshima does **not** state or even imply that the identical subunits of the *Bifidobacterium* HU protein are equivalent to the HU-1 subunit of the *E. coli* HU protein. Rather, Goshima demonstrates that *B. longum* HU has comparable N-terminal homology to both *E. coli* HU-1 and HU-2, with slightly greater homology to HU-2. Therefore, one of ordinary skill in the art would **not** deduce that *Bifidobacterium* HU has a singular promoter. Further, given that the data provided in Goshima suggest that the *Bifidobacterium* HU protein may, in fact, more closely resemble HU-2 (*see* Table 2 of Goshima, showing, in fact, that the N-terminal sequence of the *Bifidobacterium* HU protein has greater homology to the *E. coli* HU-2 protein), which has 3 promoters, than HU-1, the ordinarily skilled artisan would have **no reason** to believe that *B. longum* would have a single promoter. Moreover, Goshima is silent with respect to either regulation or expression of the HU protein in *Bifidobacterium*.

Additionally, Claret teaches that the expression of the *E. coli* HU protein is complex and highly regulated, requiring multiple promoters and multiple regulators of transcription of the HU-1 and HU-2 genes, *e.g.*, the FIS and CRP proteins. As such, it is clear that one of ordinary skill in the art would have no way of knowing what the regulatory system of the HU gene in *Bifidobacterium* would comprise given (i) the complexity of the *E. coli* HU regulatory system;

(ii) the fact that the *Bifidobacterium* HU protein exists as a homodimer and thus **must** have a different regulatory system than that of *E. coli*; and (iii) the fact that it is indeterminate whether *Bifidobacterium* HU is more like *E. coli* HU-1 or HU-2, or possibly more similar to a HU protein from a bacterial system completely different than *E. coli*.

3. Claret Does Not Teach That *E. coli* HU Proteins Are Highly Expressed

The Patent Office contends that Goshima, in addition to teaching that *Bifidobacterium* HU consists of a homodimer made from monomers like the HU-1 subunit of *E. coli* HU, teaches that (i) HU proteins are conserved in prokaryotes; (ii) HU levels in *E. coli* are relatively high; and (iii) *B. longum* HU and *E. coli* HU have similar properties. The Patent Office further alleges that Claret, in Figure 1B, teaches that the promoter for HU-1 is highly active during logarithmic growth and produces a very high percentage of the total mRNA transcripts during this phase. The Patent Office combines these alleged teachings and concludes that because one of ordinary skill in the art would expect comparable activity between the *E. coli* HU-1 and *B. longum* HU proteins, they would have expected the *B. longum* HU promoter to be highly active during logarithmic growth.

Applicants respectfully disagree and point out, as discussed above, that nowhere does Goshima correlate the *Bifidobacterium* HU protein to the HU-1 subunit of *E. coli* HU. Moreover, Applicants point out that conservation of HU among prokaryotes at the protein level does not correlate to conservation of HU at the regulatory level. Different bacteria will regulate the same protein differently and even within the same bacteria, homologous proteins can be regulated in vastly different manners, as is the case with HU-1 and HU-2 in *E. coli*. Further, Applicants point out that when discussing that *E. coli* and *B. longum* HU have similar properties, Goshima does not address the **expression** of the HU proteins in these bacteria, but only addresses phenotypic and structural properties, *e.g.*, the distance the proteins migrate in an electrophoretic field; the ability of the proteins to bind DNA; and the N-terminal sequence homology between the proteins.

Most importantly, Applicants respectfully disagree with the Patent Office's interpretation of Claret's Figure 1B. Figure 1B of Claret provides no information whatsoever regarding the level of HU-1 mRNA produced during the logarithmic phase of *E. coli* relative to the total *E. coli*

mRNA produced during logarithmic phase. Rather, Figure 1B only shows the percentage of cellular HU-1 mRNA at specific time points relative to the total amount of HU-1 mRNA (**not** the total of the mRNA transcripts produced) determined for all of the time points assessed. Therefore, it is impossible to know whether HU-1 mRNA is being produced at a high level at all based on Figure 1B. Likewise, with respect to HU-2 of *E. coli*, Figure 1B only shows the percentage of HU-2 mRNA transcribed from the 3 different HU-2 promoters at specific time points relative to the total amount of HU-2 mRNA (**not** the total of the mRNA transcripts produced) transcribed from the 3 different HU-2 promoters determined for all of the time points assessed.

4. Selection Of The Hu Promoter Was Not Obvious To Those Of Ordinary Skill In The Art

Applicants point out that, despite the fact that Goshima states that it is interested in developing a host-vector system for manipulating genes of *Bifidobacterium* (see Goshima at page 207, paragraph spanning columns 1 and 2), Goshima is completely silent regarding the possibility of utilizing a *Bifidobacterium* HU promoter in such a system. Indeed, Applicants assert that if the *Bifidobacterium* HU promoter were an obvious choice for use in a host-vector system for manipulating genes of *Bifidobacterium*, Goshima would have clearly stated that to be the case, yet did not.

Moreover, the non-obviousness of the presently claimed invention is supported by the fact that those of ordinary skill in the art, aware of the existence of HU promoters, repeatedly chose to utilize promoters other than HU to express bacterial proteins. For example, although it was known that an HU promoter existed in *Clostridium* (*see, e.g,* Goshima at page 207, left column), Brown elected to use promoters other than HU (Brown uses three separate promoters, the *E. coli* trpE promoter, the ferredoxin gene promoter, and the promoter/operator region of the *E. coli* lac operon, but does not use or contemplate the use of the *Clostridium* HU promoter whatsoever).

Applicants submit that if it were obvious to use the HU promoter in bacterial expression vector systems, then those skilled in the art, *e.g.*, Goshima and Brown, would have done so or, at a minimum, contemplated the possibility of doing so.

For at least the foregoing reasons, Applicants respectfully request that the rejection of the claims under 35 U.S.C. § 103(a), for obviousness, be withdrawn.

Conclusion

Applicants respectfully request entry and consideration of the foregoing amendments and remarks. No new matter has been introduced. The claims are believed to be free of the art and patentable. Withdrawal of the rejections and an allowance are earnestly sought.

Respectfully submitted,

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Enclosures